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Award Number: W81XWH-13-1-0252

TITLE: Disparate Vitamin D Activity in the Prostate of Men with African Ancestry

PRINCIPAL INVESTIGATOR: Larisa Nonn

CONTRACTING ORGANIZATION: UNIVERSITY OF ILLINOIS
Chicago, IL 60612

REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2015	Annual	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE
Disparate Vitamin D Activity in the Prostate of Men with African Ancestry

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0252

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Larisa Nonn

Email: lnonn@uic.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF ILLINOIS
Chicago, IL 60612

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
African American (AA) men are disproportionally affected by prostate cancer (PCa). AA men are not only at increased risk of PCa compared to American men of European descent (EA), but also are at the highest risk of aggressive PCa and death from PCa. Vitamin D3 deficiency increases PCa mortality, highlighting the importance of maintaining adequate vitamin D3 status for prostate health. Vitamin D3 is acquired in the diet or via UVB/sunlight-initiated synthesis in the skin. Cutaneous melanin absorbs UVB radiation, which leads to reduced vitamin D3 synthesis in darker pigmented skin. Consequently, ~65% of AA men are vitamin D3 deficient compared to ~20% of EA men. The level of skin pigmentation is correlated with the extent of African ancestry and serum vitamin D3 status. Besides vitamin D3 status, the activity of vitamin D3 is mediated by the vitamin D receptor (VDR) and determined by several cytochrome P450 metabolism enzymes that bioactivate/inactivate the active form of the hormone, 1,25-dihydroxyvitamin D3 (1,25D).

15. SUBJECT TERMS- nothing listed

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	8	**19b. TELEPHONE NUMBER** *(include area code)*

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Introduction

African American (AA) men are disproportionally affected by prostate cancer (PCa). AA men are not only at increased risk of PCa compared to American men of European descent (EA), but also are at the highest risk of aggressive PCa and death from PCa. Vitamin D3 deficiency increases PCa mortality, highlighting the importance of maintaining adequate vitamin D3 status for prostate health. Vitamin D3 is acquired in the diet or via UVB/sunlight-initiated synthesis in the skin. Cutaneous melanin absorbs UVB radiation, which leads to reduced vitamin D3 synthesis in darker pigmented skin. Consequently, ~65% of AA men are vitamin D3 deficient compared to ~20% of EA men. The level of skin pigmentation is correlated with the extent of African ancestry and serum vitamin D3 status. Besides vitamin D3 status, the activity of vitamin D3 is mediated by the vitamin D receptor (VDR) and determined by several cytochrome P450 metabolism enzymes that bioactivate/inactivate the active form of the hormone, 1,25-dihydroxyvitamin D3 (1,25D).

We hypothesized that the high prevalence of vitamin D3 deficiency in AA men is associated with reduced prostatic concentrations of vitamin D3, which leads to lower expression of vitamin D pathway genes and suppress pro-differentiating actions of vitamin D3 in the prostate; this ultimately abrogates the chemoprotective effects of this natural hormone and raises the susceptibility of AA men to aggressive PCa.

Keywords

Vitamin D, prostate cancer, African-American

Overall Project Summary

This research proposal brings together two well known health disparities that affect African-American men. The first is that African-American men are disproportionally affected by prostate cancer in that African-American men are not only at increased risk of prostate cancer compared to American men of European descent, but also are at the highest risk of aggressive prostate cancer and death from prostate cancer . The second disparity is the rampant vitamin D3 deficiency in the African-American population. There is a biological component to this deficiency because sun-induced vitamin D3 synthesis in the skin is significantly reduced in melanin-rich pigmented skin. Consequently, about two thirds of African-American men are vitamin D3 deficient compared to about 20% of men of European descent. It is important to maintain a healthy vitamin D3 status because vitamin D3 deficiency increases the risk of prostate cancer mortality.

Our study will directly examine the amount of vitamin D3 in the prostate tissue of a racially diverse group of patients to discern differences in African-American men. Secondly, we will investigate several mediators of vitamin D3 activity in the prostate tissue and in a novel cell culture model to identify innate molecular differences that may be present between men of European descent and in African-American men that increase susceptibility to aggressive prostate cancer in the latter group.

Key Research Accomplishments

In year one of this award we have made significant progress in both the patient sample analyses and the *in vitro* assays.

With the patient samples, in Y1 we completed African ancestry estimation and measurement of serum vitamin D metabolites in all of the patients for these study (**Figure 1**). *Of note, we changed cohorts as the patient specimens we planned to use (N=50 from collaborator Vince Freemen) became unavailable due to a freezer meltdown. We were able to acquire the necessary samples of frozen prostate tissue, serum and whole blood from 50 patients (25 AA and 25 EA) by collaborating with Dr. Peter Gann (here at UIC) and via purchase from the Cooperative Human Tissue Network (CHTN).* In our cohort, the percentage of African Ancestry in men ranged from 2-95% (**Figure 1A**), which demonstrates the diverse ethnic background of self-declared black men and underscores the value of this additional analysis in interpreting our final data sets.

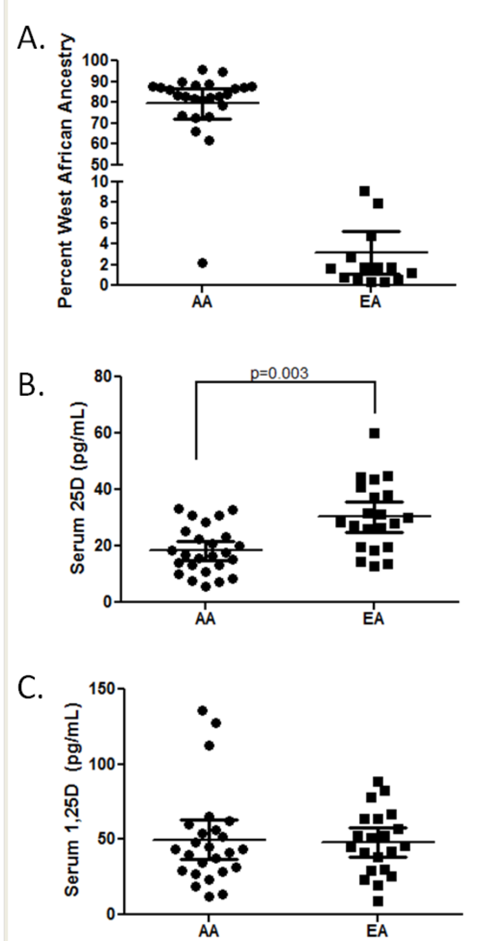


Figure 1. The ancestry and vitamin D levels in the patient cohort. A, the percentage of west African ancestry determined by SNP analysis. B and C, concentrations of 25-D (B) and 1,25D (C) in serum by UHPLC-MS-MS. Mean and 95%CI shown

Serum measurement of 25-hydroxyvitamin D (25D) is used to determine vitamin D status and the AA patients had significantly lower 25D (**Figure 1B**). The levels of 1,25-dihydroxyvitamin D (1,25D), the active hormone, were also measured and not significantly different (**Figure 1C**).

In Y2 we have completed the extraction and measurement of the vitamin D metabolites has been fully optimized in the prostate tissue. The vitamin D measurement was done in collaboration with Heartland Assays, a lab that only measures vitamin D metabolites and was started by the world renowned vitamin D expert Bruce Hollis. Heartland Labs measured all of our serum samples and in the frozen human prostate tissues. The tissue findings were quite surprising and show that the AA prostate has *higher* 1,25D compared to the EA, despite having lower 25D (**Figure 2A-B**). The levels of the vitamin D metabolism and response genes will be measured in Y3 and may explain this unexpected finding.

In Y2 the laser-capture microdissection (LCM) on the frozen prostate tissues has been completed. There is sufficient RNA from all patients to complete gene expression analysis by whole transcriptome amplification followed by PCR (**Table 1**).

Since moving to the University of Arizona in September of 2014, Dr. Kittles remains an active participant in the project. He has completed SNP analysis of all of the patients for the ancestry estimation as well as for 27 SNPs in key vitamin D response/metabolism genes (VDR, VDBP, CYP24A1, CYP27B1, etc).

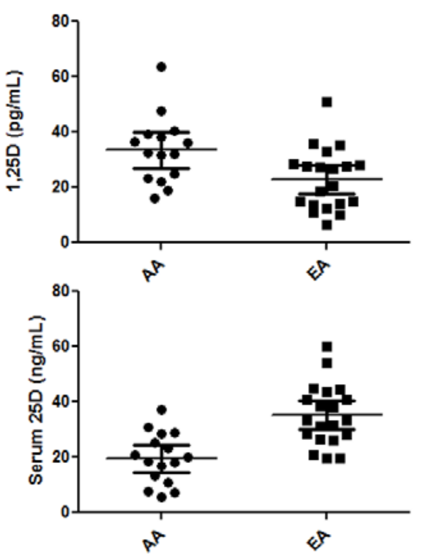


Figure 2. The prostate tissue 1,25-dihydroxyvitamin D levels in the patient cohort. Concentrations of 25-D and 1,25D in serum by UHPLC-MS-MS. Mean and 95%CI shown

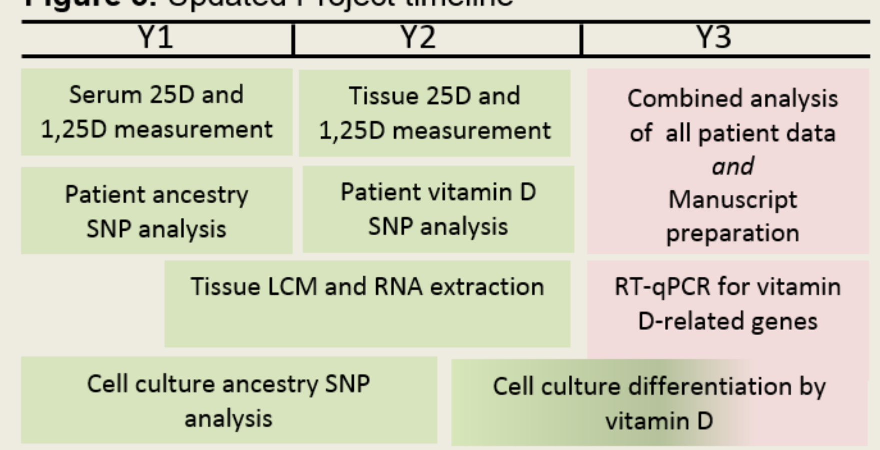
Table 1. RNA from LCM-collected benign prostate epithelium

Tissue ID	Race	RNA (ng/uL)	Total RNA (ng)	260/280
4	EA	8.59	214.75	2.09
9	AA	7.15	178.75	1.84
10	AA	7.35	183.75	1.48
11	AA	10.24	256	1.95
12	AA	21.96	549	1.73
14	AA	16.66	416.5	3.39
15	AA	4.98	124.5	1.58
24	AA	6.57	164.25	1.8
36	AA	21.22	530.5	1.69
38	EA	11.87	296.75	1.68
39	AA	27.39	684.75	1.74
40	AA	12.51	312.75	2.79
43	EA	7.4	185	2.1
45	AA	10.65	266.25	1.99
57	AA	12.35	308.75	1.89
58	EA	11.26	281.5	2.16
59	AA	9.39	234.75	2.21
62	AA	14.72	368	2.51
68	AA	9.9	247.5	1.92
74	AA	17.83	445.75	2.23
78	EA	14.24	356	2.88
80	AA	15.57	389.25	2.12
82	AA	10.05	251.25	2.49
83	AA	12.23	305.75	2.09
86	EA	9.96	249	2.2
87	AA	10.31	257.75	1.91
88	EA	7.51	187.75	1.87
89	EA	17.48	437	1.76
90	AA	11.14	278.5	1.75
WD-20645	AA	30.14	753.5	2.63
WD-20669	AA	10.49	262.25	1.81
WD-20675	AA	20.14	503.5	1.85
WD-20693	EA	7.93	198.25	2.66
WD-20696	EA	6.59	164.75	1.91
WD-25289	AA	28.12	703	1.5
WD-25292a	EA	6.16	154	1.72
WD-25295	AA	13.98	349.5	1.61
WD-25298	EA	6.98	174.5	1.66
WD-25301	EA	9.51	237.75	1.58
WD-25304	EA	6.98	174.5	1.91
WD-25307	AA	8.65	216.25	1.93
WD-25313	EA	7.9	197.5	1.91
WD-25316	EA	6.05	151.25	1.63

Conclusion

To date, we have completely analyzed prostate and serum vitamin D metabolites in the cohort. SNPs for African ancestry and 27 SNPs related for vitamin D have also been completed in the in a cohort of 50 diverse prostate cancer patients. Similar with other reports, vitamin D status in the AA patients is lower than the EA as measured by serum 25D. Unexpectedly, the tissue levels of 1,25D were higher in the AA patients compared to EA. Molecular analysis of the patient samples has begun and may reveal gene expression changes in the vitamin D metabolism genes that explain the tissue 1,25D status. We are on schedule to complete Aims 1 and 3 of the project. Aim 2, which is *in vitro*, is also on schedule. An updated proposed timeline for our current status and remainder of the project is shown in **Figure 3**.

Figure 3. Updated Project timeline



Publications, Abstracts, and Presentations

Publications:

Thesis: Farhat, Rachael S. Title: Examination of Vitamin D Disparities in African American and Caucasian Prostate Cancer Patients and Cells. URL http://hdl.handle.net/10027/19512 Publication Date 2015

Abstract and Invited Presentation: Dec 2013 American Association for Cancer Research (AACR)- The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved, Atlanta, GA."Vitamin D and prostate Cancer in African-American Men"

Abstract and Poster presentation: November 2015 Steroid Research Congress Chicago. "Vitamin D metabolites in prostate tissue and serum from African-American Men". Richards, Z, Farhat R, Kittle R, Nonn L.

Inventions, Patents and Licenses

none

Reportable Outcomes

none

Other Achievements

none

References

none

Appendices

none